Clinical aspects & interpretation of MDx results

Colin Mackenzie & Inge van Loo

Maastricht UMC+

academisch ziekenhuis Maastricht azM | Maastricht University

Why implementation of molecular diagnostic testing?

- Detection of pathogens
  - Qualitative or (Semi-) Quantitative
  - Detection of resistance markers
    - Association with culture and phenotypic methods

- Monitoring: to initiate therapy or follow up of therapy

- Typing of pathogens
  - Association of certain subtypes with disease
  - Investigation of outbreaks

Detection of pathogens:
Questions from participants: interpretation of NAAT

The sensitivity of PCR for detection of *M. tuberculosis* is generally low. Why?

What is your opinion about the use of dual-target real time PCR in a routine diagnostic laboratory compared to single-target real time PCR?

Which molecular tests are to be interpreted with caution prior to communication of the results to the clinicians?

Please explanation.

Interpretation vs clinical evidence of a MDx result

The sensitivity of PCR for detection of M. tuberculosis is generally low.

- Factors affecting the sensitivity of PCR:
  - Target – number of copies
    - Typically for *M. tuberculosis* IS6110 used, which has multiple copies and thus the sensitivity is high
  - Accesibility of DNA/RNA – mycobacteria have a thick cell wall
  - Inhibition due to extraneous DNA, other substances
- Xpert MTB/RIF: designed to be robust and specific for areas of high endemicity
- Intended as a replacement for acid fast staining and microscopy – against which it performs very well (88 % vs. 65 %)
  - Cochrane Database Syst Rev. 2014 Jan 21
  - J. Clin. Microbiol. December 2011 vol. 49 no. 12 4138-4141

What is your opinion about the use of dual-target real time PCR in a routine diagnostic laboratory compared to single-target real time PCR?

Dabisch-Ruthe *et al. BMC Infectious Diseases* 2012, **12**:163
http://www.biomedcentral.com/1471-2334/12/163

Table 2 Comparison of RVP, RespiFinder-19, RespiFinder-SMART-22 and monoplex real-time PCR with regard to the analytical sensitivity in a sample with 13 viruses

Virus	Concentration [copies/ml]	RVP				RespiFinder-19				RespiFinder- SMART-22				Monoplex real-time PCR			
		und.	1:10	1:100	1:1000	und.	1:10	1:100	1:1000	und.	1:10	1:100	1:1000	und.	1:10	1:100	1:1000
INF-A H1	1.78E+04	+	+	+	-	-	-	-	-	-	-	-	-	32.9	33.8	35.8	37.9
INF-A H3	1.42E+04	+	+	+	-	-	-	-	-	-	-	-	-				
INF-B	n. q.	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
RSV-A	3.94E+04	+	+	+	-	-	-	-	-	-	-	-	-	28.7	30.4	32.5	34.6
RSV-B	n. q.	+	+	+	-	-	-	-	-	-	-	-	-	x	x	x	x
PIV-1	1.61E+04	-	-	-	-	-	-	-	-	-	-	-	-	32.8	33.1	34.9	36.6
PIV-2	9.19E+03	-	-	-	-	+	-	-	-	+	+	+	-	29.5	31.4	33.7	36.4
PIV-3	3.84E+04	-	-	-	-	-	-	-	-	-	-	-	-	34.9	36.8	38.3	39.8
CoV OC43	n. q.	+	+	+	+	+	+	+	+	+	+	+	+	27.6	28.9	30.1	31.9
CoV 229E	n. q.	-	-	-	-	+	+	+	+	+	+	+	+	26.8	27.9	29.1	30.5
HRV	2.83E+04	+	+	+	+	+	+	-	-	+	+	+	-	31.2	33.8	36.0	38.4
AdV	3.56E+04	-	-	-	-	+	+	+	+	+	+	+	-	35.9	37.6	38.8	39.9
hMPV	1.78E+04	+	+	+	+	-	-	-	-	-	-	-	-	30.2	32.0	34.8	38.4

n. q.: not quantified; x: not performed; und.: undiluted; The dilution series were done as two-fold dilution series.

BMC Infect Dis. 2012; 12: 163

Interpretation vs clinical evidence of a MDx result

1. Association with conventional diagnostics
2. Colonisation versus infection
3. Establishing clinical cut- off
4. Interpretation of semi quantitative result for isolation measures

1. Conventional diagnostics vs rt-PCR rotavirus and gastroenteritis

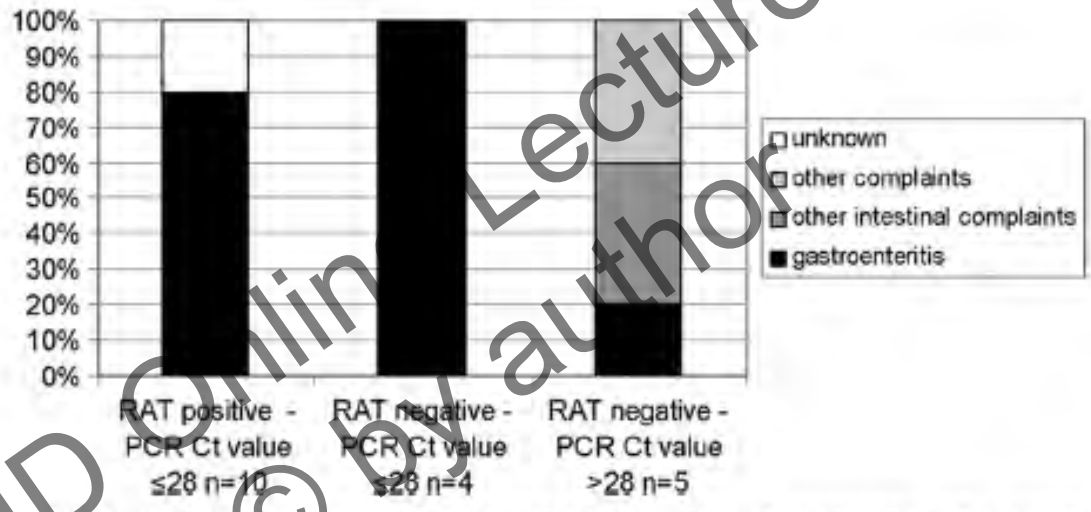


FIG. 3. Comparison of clinical symptoms of patients who were PCR positive for rotavirus and those who had either rotavirus RAT-positive detection or rotavirus RAT-negative detection. The latter group is furthermore divided into those with a low C_T value (higher rotavirus load) and high C_T values (lower rotavirus loads). In this figure, only data of patients in which rotavirus was the only virus detected by any method have been included.

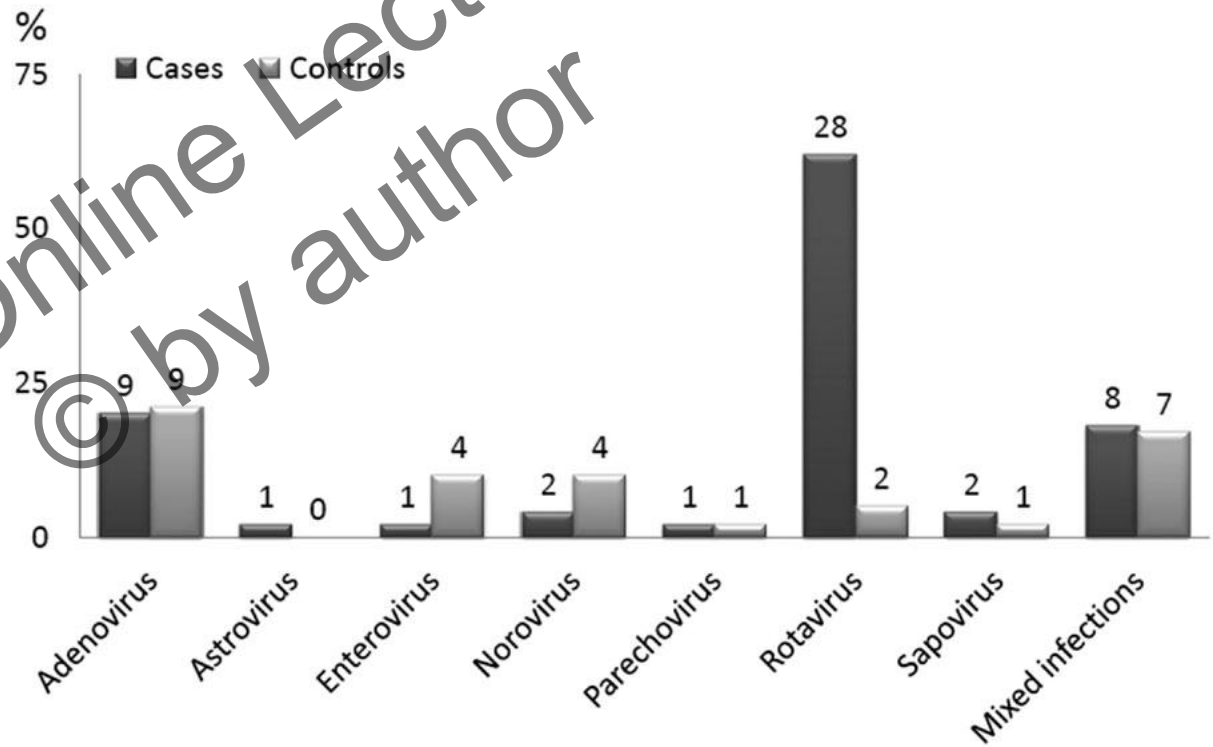
Wolffs et al., JCM, 2011

Remaining challenges: what about the presence of virus when there are no/limited symptoms?

-> case control study

Case control study: gastroenteritis in children

45 cases and 41 controls; children with and without gastroenteritis



Semi quantitative cut-off: rotavirus Ct 28

Typing adenovirus: genotypes A, C and F

2. Colonisation versus infection

- 10 times more bacteria in/on our body than human cells!

- Most commensal micro-organisms are opportunistic pathogens.

- Could quantitative molecular diagnostics be used to distinguish colonisation from infection?

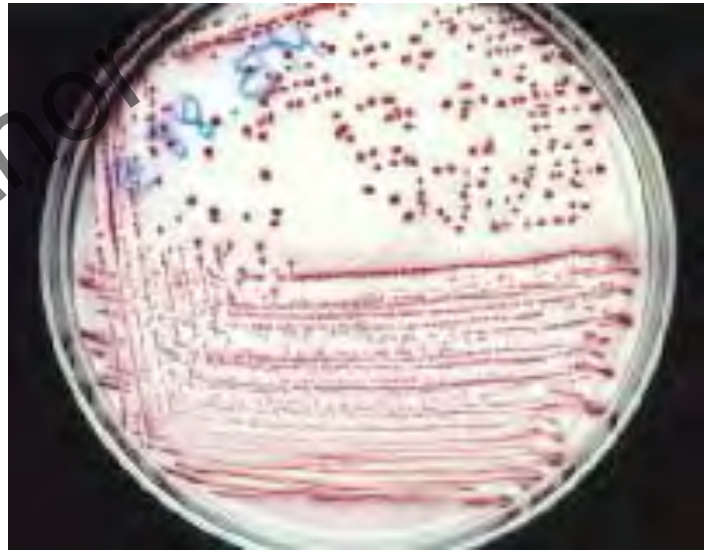
Example: Urinary tract infections

- Gold standard is semi-quantitative culture.

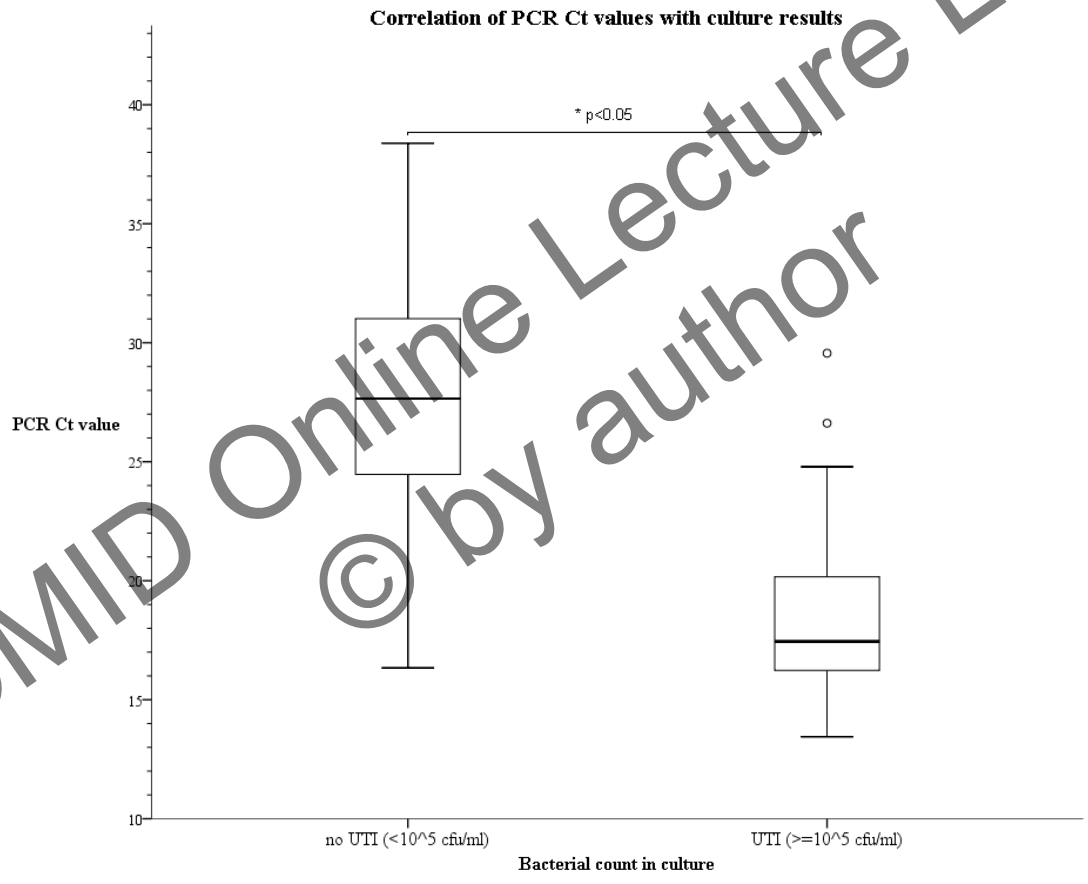
- Microbial cut-offs have been established!

- Challenge: diverse range of pathogens involved

- Solution: eubacterial 16S rDNA quantification



Molecular solution



Hansen et al., Plos One, 2013

3. Establishing clinical cut-offs for *Pneumocystis jiroveci*

- QCMD: international external quality assurance panels for Pneumocystis jiroveci

- 1 panel per year (70-120 worldwide participants)

- Clinical questionnaire regarding the use of clinical cut-offs and usage of data.

Results clinical questionnaire

	PATIENT A		PATIENT B	
	Ct 36 n(%)	**Ct 39 n(%)**	**Ct 31 n(%)**	**Ct 35 n(%)**
Positive	36 (82)	18 (41)	39 (89)	37 (84)
Negative	3 (7)	8 (18)	0 (0)	1 (2)
Ct value	3 (7)	4 (9)	3 (7)	3 (7)
Dependent on microscopy	1 (2)	2 (4.5)	0 (0)	0 (0)
Other	1 (2)	10 (23)	1 (2)	2 (4.5)
Not answered		**2 (4.5)**	**1 (2)**	**1 (2)**

Linssen et al., manuscript in preparation

Literature: clinical cut-offs for *Pneumocystis jiroveci*

Damiani et al 2013 (JCM): cut-off between 1600 and 20000 copies/microliter extracted DNA

Botterel et al 2011 (JCM): cut-off between 400 and 10000 copies/microliter extracted DNA

Matsumura et al 2011 (Mycology): cut-off between 340 en 1300 copies/milliliter sample

Muhlethaler et al 2012 (Eur Respir J): cut-off of 1450 pathogens/milliliter sample (non-HIV)

4: impact on isolation measures

Example: semi quatitative: use of load for isolation strategies.

Case of last week: 12 year old boy on ICU:

ventilated due to respiratory insufficiency.

Sputum: human Metapneumovirus Ct 18

adenovirus Ct 33

H. parainfluenzae

Question: when to finish droplet isolation? Decision could not be taken on clinical grounds, since pt was ventilated. How to interpret Ct value?

Genotypic detection of resistance markers
Questions from participants

What is the implication of direct molecular resistance testing in mycobacteria if such results cannot be reproduced by phenotypic testing, e.g. detection of rifampicin resistance in tuberculosis by Xpert MTB/RIF test that cannot be reproduced from culture?

100 % sensitivity of Xpert vs. culture

PLoS ONE 9(6): June 16, 2014

80-100 % sensitivity Line-blot Immunogenetics

98 % Hain

BMC Infect. Dis.5, 62 (2005)

Eur. Respir. J. DOI 10.1183/09031936.00061808 (2008)

What approach do the experts recommend to interpret and communicate NGS results of multi-drug resistant Gram-negative bacteria and molecular resistance information regarding aspects of treatment and hospital hygiene?

- Is there a difference between molecular detection of resistance and phenotypic detection?
  - Yes, example of ESBL, combinations of resistance mechanisms
- In which clinical circumstances will NGS be used to detect resistance?
  - WGS to detect resistance in *M. tuberculosis:* N Engl J Med. 2013 Jul 18; 369(3)
  - Screening?
- Where is the evidence concerning presence of resistance genes and expression?
  - Example ESBL and cephalosporine-resistance

Genotypic detection of resistance markers

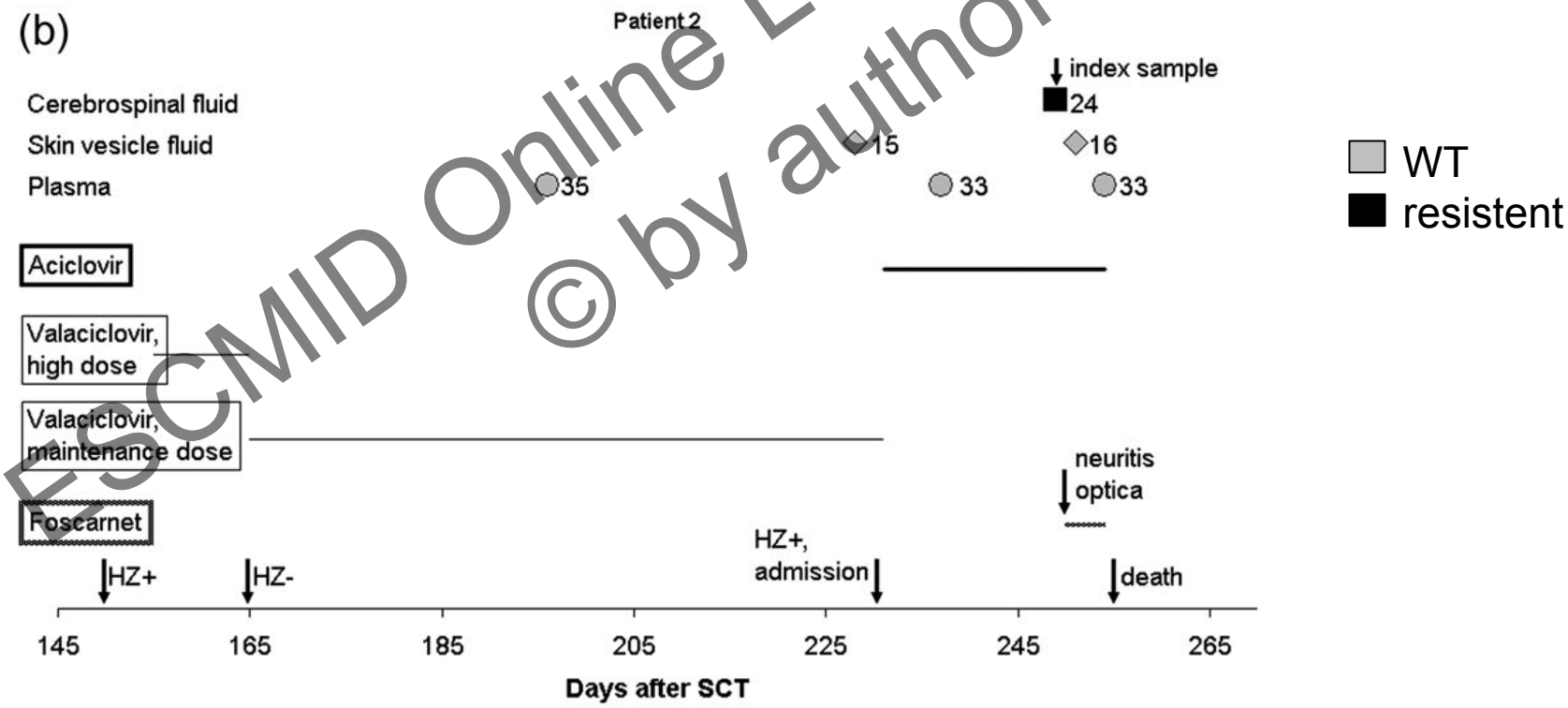
- CMV follow up load and resistance in haematologic STx ptns
- Detection of resistance mutations in UL97 / UL54 gene ~ TK and DNA polymerase gene of CMV

- Prevalence in STx patient with treatment failure is low:
- In 47 patients: 26 with treatment failure -> with resistance associated mutations.

Van Beek et al: antiviral therapy 2012

Genotypic detection of resistance markers

VZV resistance in haematologic STx: compartimentalization of resistance



Brink et al. CID 2011

Monitoring: initiation of therapy and follow up of therapy

CMV monitoring in immunocompromised -> start pre emptive therapy above a certain viral load.

Guidelines recommend:

Start gancyclovir therapy when:

Viral load is > 10,000 copies/ml in first period of reactivation

Viral load is > 100,000 copies/ml in subsequent period of reactivation or

> 1 log increase within a week and > 1000 copies/ml.

Kalpoe et al. J Clin Microbiol. 2004

Typing of pathogens: clinical relevant subtypes

- Typing clearly relates more closely to infection control than individual patient care
    - Virulence factors not generally linked to strain types
        - Exceptions: C. Difficile RT027?
- Various methods of typing
    - speed and discrimnation (rep-PCR vs. MLST or SLST)
    - cost and time (PFGE, NGS)
- WGS – examples MRSA, TB …
    - N Engl J Med 2012; 366:2267-2275
    - J Clin Microbiol. 2013 Feb; 51(2): 611–614.
- Chlamydia trachomatis:

For Proctitis: LGV (genotype D-K) versus non-LGV types (L1-L3)

- Human Papillomavirus:
- Oncongenic types e.g. 16, 18, 31 en 45.
- Non-oncogenic types e.g. 6 and 11.

Questions or comments?